Version 12-05/24

**User's Manual**

# Total PSA ELISA Kit

REF **DEIA1844**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

The Total PSA ELISA is used for the determination of total prostate specific antigen (t-PSA) in human serum or plasma samples.

## Principles of Testing

This assay is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with an antibody, directed towards an epitope of an antigen molecule (PSA). An aliquot of serum is incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After incubation the unbound E-Ab is washed off. The amount of bound E-Ab is proportional to the concentration of antigen in the sample. After adding the substrate solution, the intensity of colour developed is proportional to the antigen concentration in the sample. The measured ODs of the standards are used to construct a calibration curve against which the unknown samples are calculated.

## Reagents And Materials Provided

Each kit contains reagents sufficient for 96 determinations.

**A. Microtiterplate:**

12 modules with 8 wells each= 96 determinations

**B. 5 PSA-Standards:**

Ready-to-use reagents (0.50 mL) at the following concentrations:

1) 25 ng PSA/mL; 2) 12.5 ng PSA/mL; 3) 6.25 ng PSA/mL; 4) 3.1 ng PSA/mL; 5) 1.56 ng PSA/mL.

Preservative: Thimerosal 0.02% Kathon 0.1%.

The standards are calibrated against WHO 96/670.

**C. Zero Standard/ Sample Diluent:**

Ready-to-use reagent (10mL)

**D. Control:**

Ready-to-use reagent (0.50 mL);

for concentration see label of kit

Preservative: Thimerosal 0.02% Kathon 0.1%.

**E. PSA Conjugate:**

Ready-to-use conjugate (12 mL)

**F. TMB – Substrate:**

Ready-to-use reagent (12 mL)

Contains TMB (tetramethylbenzidine) and $H_2O_2$

**G. Stop Solution:**

Ready-to-use reagent (14 mL);

Contains sulphuric acid

## Materials Required But Not Supplied

1. Precision micropipettes (volume: 25 µL and 100 µL) with disposable tips
2. Distilled water
3. ELISA photometer with 450 nm- and 630 nm-filters
4. Timer with 60 min. range or higher
5. Microplate washer (optional)
6. Vortex or similar mixing tools
7. Container for the proper handling of waste and samples after use

## Storage

1. Store the kit and components at 2-8°C.
2. Bring to room temperature (18-25°C) at least 30 minutes before use. After use put back into the refrigerator. Avoid long time storage at room temperature.
3. Do not use the kit or components after the expiry date. For expiry date of the original packed kit see kit label.
4. Close the bottles immediately after use.
5. Store the plate incl. desiccant in the provided zip-lock pouch. Modules that are not used should always be stored under this condition.
6. Ensure that kit components do not freeze.

## Specimen Collection And Preparation

1. The preparation of serum or plasma samples is performed according to standard techniques. Serum or plasma should be prepared as soon as possible to avoid hemolysis and to improve the stability of PSA.
2. For the assay either fresh serum or plasma samples can be used. If not used immediately they can be stored at 2-8°C for 1 week. In case of longer storage, freeze at -20°C. A repeated freezing and thawing of samples should be avoided.

Note:

1) Highly lipemic or hemolytic samples can give incorrect analytical results.
2) Samples must be free of microbial contaminations.
3) Samples containing high titers of rheumatoid factor and human anti-mouse antibodies (HAMA) could give erroneous results.

## Assay Procedure

Note: It is highly recommended to perform all measurements as duplicates. An independent standard curve should be made for each series of measurements. For best results it is important that the solutions are always added to the wells in the same order to minimize variations in incubation times.

1. Prior to use bring all reagents, standards, controls, and samples to room temperature (18-25°C).
2. Check that all components are not expired and take care that bottles and plate (inclusive pouch) are not damaged.
3. Format the required microplate wells. Keep in mind that all measurements should per performed as duplicate. Document position of wells and respective samples, standards and controls to ensure later identification. Put any unused microwell modules back into the zip lock bag with the desiccant, seal bag and store at 2-8°C.
4. Pipette 25 µL of standards, controls or samples into each well. Samples with an expected PSA value higher than 25 ng/ml should be diluted with the sample diluent.
5. Incubate 5 min at room temperature (18-25°C).
6. Add 100 µL of PSA conjugate into each well.
7. Mix by moving plate on the table (10sec).
8. Incubate 1 h at room temperature (18-25°C).
9. Remove solution from the wells by aspirating the liquid or by decanting it. If decanting, tap plate on adsorbent paper to remove residual liquid.
10. For washing fill plate with distilled water and wait 15 sec before removing the distilled water; wash 5× to 6×. We recommend the following procedure: wash wells 6-times with 250 µL/well distilled water. Preferably use an automated washing procedure. If washing manually take care that the washing solution remains in each well for the same time. This is necessary to receive lowest possible CV-values.
11. Pipette 100 µL TMB-substrate solution into each well.
12. Incubate 20 min at room temperature (18-25°C).
13. Add 100 µL/well stop solution (same order as substrate solution).
14. Read absorbencies (OD) at 450 nm (blanking 630nm).

## Quality Control

It is recommended that internal controls are used in every assay in duplicate. Control results should be within established ranges and should preferably represent low, medium, and high concentrations.

## Calculation

1. Calculate the mean absorbance for each duplicate.
2. Subtract the absorbance value of the zero standard from the mean absorbance values of standards, control and samples.
3. Draw the standard curve on lin-lin or log-log graph paper by plotting absorbance values of standards against appropriate PSA concentrations or use a proper software of the ELISA reader used.
4. Read off or calculate the PSA concentrations for the control and the samples.

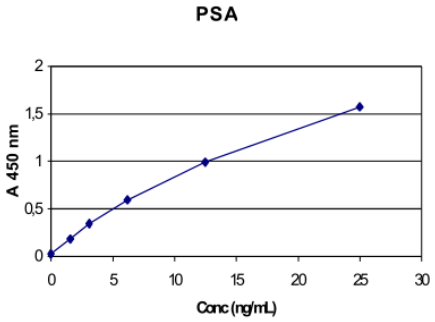
## Interpretation Of Results

1. The OD 450 nm of the blanking well is lower than 0.150. Higher values indicate a chromogen/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
2. The OD 450 nm of the highest standard (25 ng/mL) must be higher than 0.700. Lower values indicate kit or control decay. In such a case, check the expiry date of the kit before repeating the assay.
3. The control provided should not differ by more than 15% from the concentration stated on the label of the vial if run at least in duplicate.
4. Worksheet and standard curve of typical assay: Not to be used for calculation of actual test results.

Example:

| Wells | Identity | A 450 nm | | Conc. ng/mL |
|---|---|---|---|---|
| 1-2 | St 0ng/mL | 0.022 | 0,023 | |
| 3-4 | St 1,56ng/mL | 0.178 | 0.180 | |
| 5-6 | St 3,10ng/mL | 0.337 | 0.342 | |
| 7-8 | St 6,20ng/mL | 0.611 | 0.568 | |
| 9-10 | St 12,50ng/mL | 0.990 | 0.984 | |
| 11-12 | St 25,00ng/mL | 1.574 | 1.562 | |
| 13-14 | control | 0.421 | 0.400 | 3.98 |

Lot 30902.2




Note: The absolute OD values for the standards might vary due to temperature influences or age of the conjugate. As long as the OD values form a standard curve and remain within the specifications and the control shows the expected value, results for unknown PSA samples are valid.

## Precautions

1. ELISA kits are only for inresearch use by professionals.
2. Serum and plasma samples should be treated as potentially infectious materials. Wear gloves and proper laboratory attire when handling sample materials. Do not eat, drink or smoke in areas where specimen or kit reagents are handled. Do not pipette with the mouth. In case of skin contact, wash with a germicidal soap and copious amounts of water. Seek medical advice if indicated.
3. The PSA standards and controls are of human origin. They have been tested and confirmed negative for HIV, HBsAg and HCV. However, all standards should be treated as potential biohazards.
4. Due to the potentially infectious character of samples and kit components all materials that have come in contact with these materials should be sterilized and disposed of according to local regulations. This also includes the liquid waste.

5. The assay reagents contain preservatives, TMB, $H_2O_2$ or sulphuric acid and may be harmful if ingested. A direct skin or mucosa contact should be avoided. In case of skin contact, wash thoroughly with water and seek medical attention if required.
6. The stop solution contains $H_2SO_4$. Since the $H_2SO_4$ used to terminate the color reaction is corrosive, the instrumentation employed to dispense it should be thoroughly cleaned after use.
7. Do not interchange reagents from different LOT# or different suppliers.
8. Avoid reagent or sample carry-over by using fresh tips for solutions and samples.
9. Do not use test kit if zip lock pouch or bottles have been damaged.